Oxidation of Ascorbic Acid by a Trivalent Copper Complex

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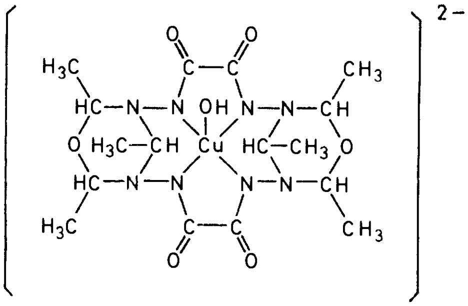
The kinetics of the oxidation of ascorbic acid (AsH_2) by trivalent copper complex (1) were studied under anaerobic conditions over the pH range 2—7. A bell-shaped pH–rate profile was obtained indicating a maximum at pH 4.5 The active species near the maximum are the hydrated Cu^{III} complex and the ascorbate ion.

RECENTLY, Hamilton *et al.* have suggested the importance of trivalent copper for monocopper enzymes such as galactose oxidase.[1] Several trivalent copper complexes are known, *i.e.* bis(biuret)-,[2] bis(oxamide)-,[2] and tetraglycine-copper(III),[3] *etc.* However, the former two are highly insoluble in water and the latter is water-soluble but unstable. Therefore, they are not suitable for detailed study of redox reactions in aqueous solution. However, a stable and water-soluble Cu^{III} complex (1) has been prepared[4a] and the structure determined by *X*-ray analyses.[4b,c] In addition, water-stable Cu^{III} chelates of diglycylethylenediamine and bis-(*N*-acetylethylenediamine) have also been reported, and the oxidation of ascorbic acid and catechol has been mentioned briefly.[5]



(1)

Since we are interested in the model reactions of metalloenzymes, we studied the kinetics of the oxidation of ascorbic acid (AsH_2) by the Cu^{III} complex (1).

RESULTS AND DISCUSSION

Stoicheiometry of Oxidation.—The stoicheiometry was first determined for the condition $[AsH_2] \ll [Cu^{III}]$. The amount of consumed (reduced) (1) could be readily determined by the decrease in its absorbance at 540 nm (ε 29 500 l mol^{-1} cm^{-1}).[4a] As shown in Table 1, the amount of (1) consumed was approximately twice the molar equivalent of AsH_2 in each of four runs. In other experiments, the possible formation of Cu^I was examined by addition of cuproin (2,2′-biquinoline) but no complex formation was observed for the condition of $[AsH_2] \ll [Cu^{III}]$. Cuproincopper(I) was detected in the presence of an excess of AsH_2, the yield of complex being quantitative for the condition $[AsH_2]/[Cu^{III}] > 2$. These results indicate the stoicheiometries (1) for $[AsH_2] \ll [Cu^{III}]$ and (2) for $[AsH_2] \gg [Cu^{III}]$.

TABLE 1

$[Cu^{III}]$ consumed in the reaction with AsH_2 after 30 min at 25 °C

	$10^5[Cu^{III}]$/M	
$10^5[AsH_2]$/M	Initial	Consumed
0	5.05	0.05
1	5.05	2.15
1.5	5.06	3.11
2	5.09	4.06
2.5	5.19	4.89

pH 4.66 (0.1M-acetate; *I* 0.1).

$$2Cu^{III} + AsH_2 \longrightarrow 2Cu^{II} + As + 2H^+ \quad (1)$$

$$Cu^{III} + AsH_2 \longrightarrow Cu^{I} + As + 2H^+ \quad (2)$$

The competitive reactions (3)—(8) are conceivable.

$$Cu^{III} + AsH_2 \longrightarrow Cu^{II} + AsH\cdot + H^+ \quad (3)$$

$$Cu^{III} + AsH\cdot \longrightarrow Cu^{II} + As + H^+ \quad (4)$$

$$Cu^{II} + AsH_2 \longrightarrow Cu^{I} + AsH\cdot + H^+ \quad (5)$$

$$Cu^{II} + AsH\cdot \longrightarrow Cu^{I} + As + H^+ \quad (6)$$

$$Cu^{III} + Cu^{I} \longrightarrow 2Cu^{II} \quad (7)$$

$$Cu^{III} + AsH_2 \longrightarrow Cu^{I} + As + 2H^+ \quad (8)$$

Faster rates for reactions (3) and (4) than for (5) and (6), or alternatively a rapid proportionation reaction (7), may give rise to stoicheiometry (1). For the formation of Cu^I in reaction (2), two mechanisms, stepwise [equations (3)—(6)] and one-step [equation (8)], are conceivable. However, the one-step mechanism seems highly unlikely on the following grounds. A square planar ligand geometry such as that for (1) is highly unusual for Cu^I (d^{10} systems) which tend to be tetrahedral.[6] The Cu^{III} complex of tetradentate diglycylethylenediamine is known not to be reduced to the Cu^I state, whereas the Cu^{III} complex of bidentate bis-(*N*-acetylethylenediamine) is reduced to the Cu^I complex, in which a change of the ligand geometry from square planar to tetrahedral is quite easy.[5] From the same reason, the reduction of Cu^{II} to Cu^I [equations (5) and (6)] is likely to occur through the dissociated Cu^{II} ion rather than through a square planer complex. In addition, uncomplexed Cu^{II} ion is known to be reduced to Cu^I by AsH_2.[7] The instability of the Cu^{II} state of complex (1) in acidic media (see later) accords with this interpretation.

Kinetics of Cu^{III} *Reduction.*—Rates were followed by monitoring the decrease of (1) (540 nm) in the presence of a large excess of AsH_2, giving pseudo-first-order rate constants ($k_{obs.}$) up to more than two half-lives. These

$k_{obs.}$ values were then observed to increase linearly with an increase of AsH_2 concentration at two pH values (2.50 and 6.45). The results are shown in Table 2 and Figure 1. These observations indicate that the rate of oxidation is first order in both (1) and AsH_2, and can be represented by equations (9) and (10). The apparent second-order rate constant, k_2', was pH dependent as described below. The subscript T refers to total ascorbic acid and total Cu^{III} species.

TABLE 2

Rate constants for the oxidation of ascorbic acid with Cu^{III} complex (1) at 25 °C

pH [a]	$10^5[Cu^{III}]$/M	$10^3[AsH_2]$/M	$10^3k_{obs.}/s^{-1}$
2.30	1.86	1.15	2.71
2.50	3.00	1.3	5.15
2.50	3.00	5.2	15.8
2.50	3.00	9.1	27.3
2.50	3.00	13.0	38.7
3.43	1.86	1.15	8.33
3.84	1.86	1.15	13.5
4.14	1.86	1.15	15.3
4.44	1.86	1.15	17.3
4.71	1.86	1.15	17.7
4.96	1.86	1.15	14.3
5.55	1.86	1.15	5.03
6.45	3.00	1.3	1.38
6.45	3.00	2.6	2.58
6.45	3.00	5.2	5.42
6.45	3.00	9.1	10.3
6.45	3.00	13.0	14.5
6.56	1.86	1.15	1.43

[a] pH 2.3—2.5, 0.1M-citrate; pH 3.43—5.55, 0.1M-acetate; pH 6.45—6.56, 0.1M phosphate; I 0.1 (KCl).

$$-d[Cu^{III}]/dt = k_2'[AsH_2]_T[Cu^{III}]_T \qquad (9)$$

$$k_{obs.} = k_2'[AsH_2]_T \qquad (10)$$

pH-Rate Profile.—The pH–rate profile for the k_2' values is shown in Figure 2. A bell-shaped profile was obtained with a maximum at pH 4.5, which is very close to the value $(pK_a + pK_h)/2 = 4.52$, where pK_a and pK_h are the dissociation constants of ascorbic acid and hydrated (1) (Cu_{aq}^{III}), respectively. The reactions (11)—(17) appear to account for this pH–rate profile. The apparent second-order rate constant, k_2', can then be represented by equation (18). The best fits of experimental k_2' values to this equation gave the theoretical solid line of Figure 2 with the corresponding values of k_1, $(k_2 + k_3K_a/K_h)$, and k_4 of 2.4, 162, and 0.5 dm³ mol⁻¹ s⁻¹, respectively.

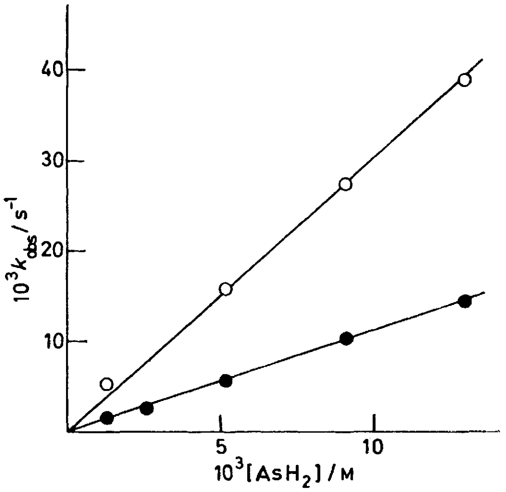


FIGURE 1 Plots of $k_{obs.}$ *versus* ascorbic acid concentration: ○, at pH 2.50; ●, at pH 6.45

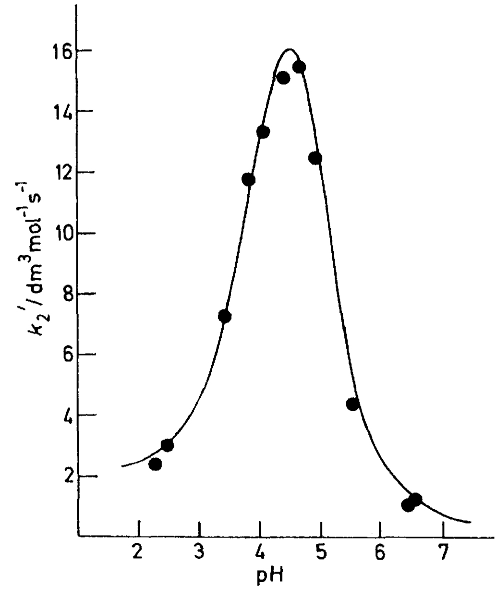


FIGURE 2 pH–Rate profile: ●, experimental points; ——, theoretical line

$$AsH_2 \overset{K_a}{\rightleftharpoons} AsH^- + H^+ \quad pK_a = 4.17^{8} \qquad (11)$$

$$Cu_{aq}^{III} \overset{K_h}{\rightleftharpoons} Cu^{III}OH + H^+ \quad pK_h = 4.87^{4a} \qquad (12)$$

$$Cu_{aq}^{III} + AsH_2 \xrightarrow{k_1} Cu^{II} + radical \qquad (13)$$

$$Cu^{III}OH + AsH_2 \xrightarrow{k_2} Cu^{II} + radical \qquad (14)$$

$$Cu_{aq}^{III} + AsH^- \xrightarrow{k_3} Cu^{II} + radical \qquad (15)$$

$$Cu^{III}OH + AsH^- \xrightarrow{k_4} Cu^{II} + radical \qquad (16)$$

$$[Cu_{aq}^{III}]_T + radical \xrightarrow{fast} Cu^{II} + As \qquad (17)$$

$$k_2' = \frac{k_1[H^+]^2 + (k_2K_h + k_3K_a)[H^+] + k_4K_aK_h}{(K_h + [H^+])(K_a + [H^+])} \qquad (18)$$

This reaction scheme assumes that the hydroxide-free, hydrated Cu_{aq}^{III} complex and ascorbate anion (AsH^-) are the active species near the pH maximum. That is, among the rate-limiting steps the fastest is k_3 [equation (15)]. However this rate is not directly observable at present, although values of k_1 and k_4 could be obtained from the two edges of the bell-shaped plot. Nevertheless, an estimation may be possible as follows. As mentioned above, equation (19) was obtained by analysis of the bell-shaped rate profile. Here, the k_2 value should be smaller than both k_1 (2.4 dm³ mol⁻¹ s⁻¹) and k_4 (0.5 dm³ mol⁻¹ s⁻¹) because the activity should be in the order $Cu_{aq}^{III} > Cu^{III}OH$ and $AsH^- > AsH_2$. Accordingly k_3 is estimated as 32 dm^3 mol^{-1} s^{-1}.

$$k_2 + k_3K_a/K_h = k_2 + 5.01\ k_3 = 162\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1} \qquad (19)$$

The intermediacy of ascorbate radical is again postulated. This appears to be reasonable in view of its formation in the related oxidation of AsH_2 with Fe^{III} complex.[9]

Fate of Copper Complex.—After the reaction, oxygen was bubbled through the mixture, but regeneration of the starting complex (1) was not observed regardless of the proportions of (1) or AsH_2. This indicates decomposition of the complex under our conditions. The corresponding Cu^{II} complex was prepared under alkaline, anaerobic conditions and then exposed to air to form the Cu^{III} complex (1). This is stable in acidic media, but when reduced to the Cu^{III} state, it dissociates and the dissociated ligand decomposes rapidly (see Experimental section).

Thus complex (1) cannot be used as a catalyst. This is disappointing for further studies of biological redox systems.

EXPERIMENTAL

Materials.—Ascorbic acid (Wako; special grade) was used without further purification. Stock solutions of ascorbic acid were prepared in deionized, distilled water. Complex (1) was prepared and purified by column chromatography following the literature procedures.[4] The absorption spectra at pH 3 and 8 were in agreement with those reported.

Determination of pK_a *of* (1).—pH-Dependent spectral changes of (1) over the range pH 3—8 were observed to give a maximum at 310 nm (at higher pH) with isosbestic point near 332 nm which allowed the determination of the pK_a in the usual manner.

Determination of Cu^I *Ion by Cuproin.*[10]—Ascorbic acid–complex (1) mixture was pipetted out and mixed with isoamyl alcohol (4 ml) containing 0.02% cuproin. After vigorous shaking, the isoamyl alcohol layer was separated and used for spectrophotometric determination of Cu^I by measuring the absorbance of cuproincopper(I) complex, $\lambda_{max.}$ 546 nm (ε 6 700 l mol^{-1} cm^{-1}). A control experiment was carried out in the absence of ascorbic acid.

Kinetic Measurements.—In a Thunberg cuvette was placed a buffer solution (3 ml) containing (1) in the lower and the desired amount of AsH_2 solution in the upper vessel. Nitrogen saturated with water vapour was bubbled into the lower and upper parts of the cuvette for 30 and 5 min, respectively. After equilibration at 25 °C, the reaction was initiated by mixing and the decrease of Cu^{III} absorbance (540 nm) was followed with a Shimazu 200 u.v. spectrophotometer. The pseudo-first-order rate constants were calculated in the usual manner. The pH was measured with a Hitachi–Horiba F-7DE pH meter after the reaction and confirmed to be the same as that of the starting buffer solution within $\pm$0.04 unit.

Stability of the Cu^{II} *Complex.*—An orange solution of Cu^{II} complex, $\lambda_{max.}$ 460 nm (ε 190 l mol cm^{-1})[4a] was stable when prepared under anaerobic, basic conditions and was oxidized to Cu^{III} complex (1) when exposed to air. A solution (30 μl) of Cu^{II} complex was added anaerobically in a dry-box to a buffer solution (3 ml) of varied pH (7.07, 4.52, 1.22), kept for 10 min, and then exposed to oxygen bubbling. The yield of Cu^{III} complex (1) was *ca.* 40 (pH 7.07), 8 (4.52), and <4% (1.22).

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